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How Selective Is the Transporter Associated with Antigen Processing?

Minireview

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Recognition of foreign and self-antigens by T cells requires the interaction of the T cell receptor with major histocompatibility complex (MHC) class I or class II molecules on the surface of the antigen-presenting cell. The specificity of the interaction is provided by the antigenic peptides that are bound to the MHC molecules. Generally, MHC class I molecules present peptides that are derived from endogenous protein sources, while MHC class II molecules present peptides from exogenous proteins. Many of the peptides presented by class I are derived from cytosolic proteins, and therefore these peptides must cross a lipid bilayer membrane in order to assemble with newly synthesized class I molecules in the lumen of the endoplasmic reticulum (ER) (see Figure 1). In contrast, MHC class II–associated peptides are not subjected to a similar requirement, as internalized antigen in endosomes or lysosomes is topologically equivalent to the class II molecules. To get peptides into the lumen of the ER, the cell has adapted a transport molecule from the large family of transport proteins called ATP-binding cassette (ABC) transporters (for review see Higgins, 1992). Examples of well-characterized ABC transporters include P-glycoprotein (Chen et al., 1986; Gros et al., 1986), the cystic fibrosis transmembrane conductance regulator (CFTR) (Riordan et al., 1989), and the STE 6 transporter in yeast (Kuchler et al., 1989). The peptide transporter, called the transporter associated with antigen processing (TAP), is composed of two homologous proteins termed TAP1 and TAP2 (Trowsdale et al., 1990; Spies et al., 1990; Deverson et al., 1990; Monaco et al., 1990). Together, the TAP1/TAP2 heterodimer forms a transport molecule that conserves the hallmarks of ABC transporters, i.e., the possession of approximately twelve membrane-spanning domains and two hydrophilic ATP-binding domains (see Figure 2). For ABC transporters, the hydrolysis of ATP is coupled to substrate transport.

The critical role of TAP in class I antigen processing was established through the study of mutant cells that either lacked the TAP proteins (DeMars et al., 1985; Salter and Cresswell, 1986), or expressed a nonfunctional form of TAP1 or TAP2 (Ljunggren et al., 1989). These cells possess impaired class I antigen processing and express low levels of class I molecules on their cell surface. It was theorized that the transporter proteins missing from these cells were responsible for the transport of antigenic peptides from the cytosol into the ER for stable assembly with class I. It was shown that this was indeed the case when the introduction of TAP genes into the mutant cells restored the normal class I processing phenotype (Spies and DeMars, 1991; Arnold et al., 1992; Powis et al., 1991; Attaya et al., 1992), and when it was revealed that TAP directly transports antigenic peptides into the ER (Neefjes et al., 1993; Shepherd et al., 1993; Androlewicz et al., 1993).

Two primary functional assays grew out of the early studies on TAP. One assay measures peptide transport across the ER membrane in streptolysin O–permeabilized cells (Neefjes et al., 1993; Androlewicz et al., 1993) or microsomal preparations from mice or insect cells that express recombinant TAP (Shepherd et al., 1993; Meyer et al., 1994). The second assay measures peptide binding to TAP (van Endert et al., 1994). The key difference between these two assay systems is that peptide transport requires ATP, whereas peptide binding does not require ATP, and to measure peptide binding ATP must be excluded. An important feature of the peptide transport assay is the use of glycosylatable peptides to measure directly the transport of peptides into the ER. This technique (pioneered by Neefjes et al., 1993) takes advantage of the natural glycosylation machinery located in the ER of cells. Peptides that contain an N-linked glycosylation site (NXS or NXT), either naturally or engineered, become glycosylated upon entry into the ER. This will occur as long as there is at least 1 aa C-terminal to the glycosylation site. This provides a direct readout for the transport of radiolabeled peptides simply by measuring the level of peptide glycosylation through binding to concanavalin A–Sepharose. How does the peptide transport assay relate to the peptide binding assay in terms of TAP function? The data suggest that peptide binding and peptide transport are closely coupled. Indeed, when the same set of peptides were tested in both assay systems the results were remarkably similar (Androlewicz and Cresswell, 1994, van Endert et al., 1995). Therefore, even though peptide transport by TAP can be broken down into two steps, i.e., peptide binding and peptide translocation, in functional assays the two steps are indistinguishable.

With the role of TAP firmly established, the stage was set for investigation into the substrate specificity of TAP. The main issue here is to what degree does TAP select peptides that will be accessible to class I molecules. The specificity issue can be further divided into two types: one is specificity for peptide length and the other is specificity for peptide sequence. The data has revealed a clear peptide length specificity, with TAP preferring peptides in the 8–12 aa range. TAP peptide sequence specificity appears to vary between species, with human TAP being the most promiscuous, mouse TAP the least promiscuous, and rat TAP lying somewhere in between. The initial TAP sequence specificity studies focused on the effect that the C-terminal amino acid of the peptide has on selection by TAP. Mouse TAP possesses a clear preference for peptides that contain

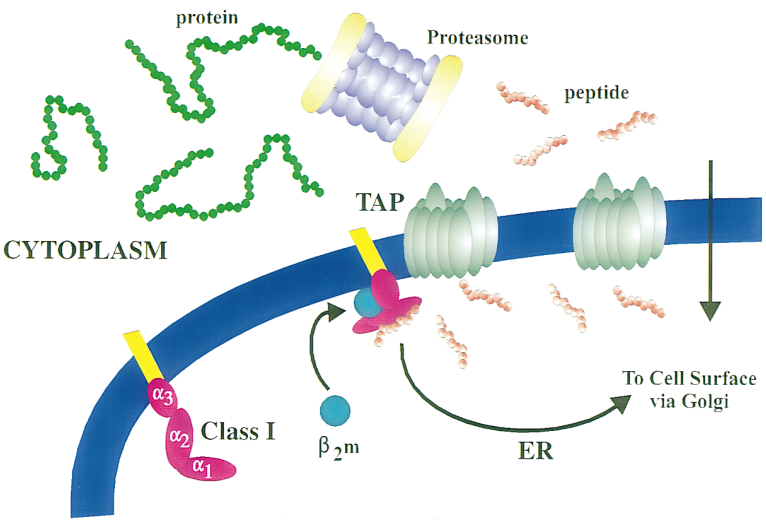

Figure 1. Model of the MHC Class I Antigen Processing Pathway

Protein antigens are degraded into small peptides in the cell cytosol by the proteasome complex. The peptides are transported into the lumen of the ER by the transporters associated with antigen processing (TAP) where they assemble with newly synthesized MHC class I heavy chains and β_2-microglobulin (β_2m). Once assembled, the stable complex of heavy chain, β_2m, and peptide leaves the ER and is expressed on the cell surface. Recent data suggest that the class I heavy chain and β_2m associate with TAP prior to peptide loading, and that the binding of peptide releases the complex from TAP. (Illustration by E. Hughes and M. Honey.)

hydrophobic (aliphatic and aromatic) C-terminal residues (Schumacher et al., 1994), whereas human TAP does not possess a clear preference for any particular C-terminal residue (Momburg et al., 1994a). Rat TAP possesses both the hydrophobic as well as the nonspecific C-terminal residue specificity (Heemels et al., 1993; Heemels and Ploegh, 1994).

The rat transporter is unique in the sense that different alleles of TAP have functional consequences. Two different forms of the rat transporter are based on two allelic versions of the rat TAP2 molecule. They differ by approximately 25 aa, which are spread throughout the membrane-spanning domains. The rat TAP1/TAP2^a (cim^a) heterodimer resembles human TAP in that it does not have a preference for a particular amino acid at the C terminus, while the rat TAP1/TAP2^u (cim^b) heterodimer resembles mouse TAP in that it prefers peptides with hydrophobic residues at the C terminus. This difference results in distinct pools of peptides available for binding to the rat class I molecule RT1A^a. Because the RT1A^a molecule prefers peptides that possess basic residues such as arginine at the C terminus (Powis et al., 1996), it only becomes efficiently loaded in the presence of the rat TAP1/TAP2^a complex. The allelic forms of human and mouse TAP have not yet been shown to have any significant effect on peptide loading.

Several TAP functional studies were performed that analyzed the role of amino acids at or near the N terminus of the peptide. In one study (Momburg et al., 1994b), peptides with amino acid substitutions at the N terminus of an 8-mer peptide associated with mouse MHC class I molecules were analyzed for peptide transport by TAP. It was concluded that amino acid substitutions at the N terminus had only a minor effect on peptide transport across the three species tested. However, this study was restricted by the number of different epitopes used and the placement of the amino acid substitutions. In another study (Androlewicz and Cresswell, 1994), the TAP transport capacity of a panel of peptide variants, each derived from a natural epitope found associated with MHC class I molecules, was analyzed. The conclusion drawn was that human TAP was promiscuous with regard to the sequence of the peptides that it bound and transported. This conclusion was reached because the majority of peptide variants, including ones made up of predominantly alanine residues, were transported well by TAP. However, this study was restricted in that variants from only three distinct class I epitopes were used and therefore the results may have been skewed toward peptides that bind to class I and are transported well by TAP. In a third study, which first described the peptide binding assay for recombinant TAP in insect cell microsomes (van Endert et al., 1994), it was shown that the affinity for TAP of a set of peptides in the 9–11 aa range varied widely. However, the number of peptides used in this study was limited and no definite conclusions on the substrate specificity of TAP could be drawn. All studies so far are in agreement with the observation that blocked N- or C-terminal residues inhibit peptide transport by TAP (Momburg et al., 1994a; Schumacher et al., 1994).

More definitive conclusions can be drawn from the length specificity studies that have been performed on TAP. All studies are in close agreement that the minimal length required for peptide transport by TAP is 7–9 aa (Schumacher et al., 1994; Androlewicz and Cresswell, 1994; Momburg et al., 1994b; van Endert et al., 1994; Heemels and Ploegh, 1994). However, the maximal length of peptides that can be transported by TAP is not as clear cut. It was shown that at least one peptide of 24 aa in length can effectively compete for reporter peptide transport (CLIP, Androlewicz and Cresswell, 1994). Generally, as peptide length increases beyond the 8–12 aa optimal length (up to 40 aa so far tested), the capacity of the peptides to be transported or to compete for peptide transport by TAP decreases (Androlewicz and Cresswell, 1994; Momburg et al., 1994b; Heemels and Ploegh, 1994). Of course, in some cases it is hard to distinguish between the effects of peptide length and peptide sequence. In TAP peptide binding studies (van Endert et al., 1994) it was shown that peptides in the 9–16 aa range have high affinities for TAP, while longer peptides have lower affinities. Overall, the data on peptide length suggest that peptides longer

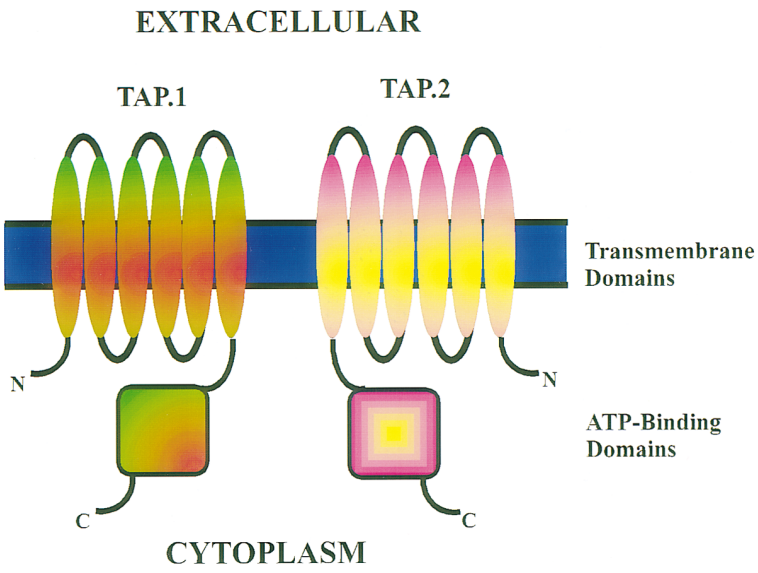

Figure 2. Structural Organization of TAP

The TAP transporter is a heterodimer of two related proteins called TAP1 and TAP2. The heterodimer consists of approximately twelve hydrophobic membrane-spanning regions, and two hydrophilic ATP-binding domains. The TAP1/TAP2 dimeric structure is required to form a functional transporter, i.e., TAP1 and TAP2 homodimers are not functional. (Illustration by M. Honey.)

than 12 aa can be transported by TAP, but at a reduced efficiency. Interestingly, there appears to be a difference in peptide length preference between the rat cim^a and cim^b forms of TAP. The cim^a allele, which is the least selective at the C terminus, is more selective with regard to peptide length (not allowing peptides longer than 10 aa to pass) than the cim^b allele (Heemels and Ploegh, 1994).

A further step in the elucidation of TAP sequence specificity has been taken with the recent identification of a peptide-binding motif for human TAP (van Endert et al., 1995). The investigators utilized the microsomal peptide binding assay to measure the affinity for TAP of more than 250 peptides and peptide variants. From these data, the investigators were able to identify a putative peptide-binding motif for TAP. The nature of the motif illustrates how the binding site for TAP differs from the peptide-binding site of class I molecules. Particularly, there are no single amino acids that serve as primary anchor residues as described for the class I–associated peptide motifs (for review see Rammensee et al., 1995). Rather, there are sets of amino acids at the N terminus of the peptide, which TAP prefers. It appears that TAP prefers strongly hydrophobic residues in position 3 (P3) of the peptide, and hydrophobic or charged residues in P2. However, aromatic or acidic residues in P1, as well as proline in P1 or P2, have strong negative effects on peptide affinity. Therefore, even though the TAP motif is not as stringent as the HLA motifs, it appears to be biased toward peptides that bind to HLA molecules, as the specificity for both is dependent upon residues at the N terminus. In fact, the investigators show that the percentage of peptides with very low TAP affinities is significantly reduced in a group of naturally processed peptides versus a group of random peptides. Peptide-binding motifs have not yet been identified for the mouse or rat transporters; however, a recent study has revealed that a prolyl residue in P3 has a strong negative effect on peptide transport by mouse TAP (Neisig et al., 1995).

While the newly defined motif for human TAP suggests that TAP transports peptides that are predisposed to binding to class I molecules, caution should be taken in the formation of any hard and fast rules. First, the overall affinity of a given peptide for TAP is determined by a sum of the positive and negative interactions, and any given negative interaction may be overcome by a stronger positive interaction elsewhere in the peptide. Second, a significant number of HLA class I–binding motifs are not consistent with the TAP peptide-binding motif. Namely, the motifs for HLA-A1, HLA-B7, HLA-B8, and other HLA-B and HLA-C alleles possess detrimental amino acid residues as primary anchor sites. There is a particular propensity for the use of proline in position 2 as a primary anchor among the HLA-B alleles. Of course, one can argue that longer peptides that contain the class I–binding epitopes are transported well by TAP and then once inside the ER are trimmed to the appropriate size. In this case, it remains unclear as to why the cell would impose another level of complexity to the processing of certain class I epitopes. Third, it should not be concluded that because a peptide possesses a low affinity for TAP the peptide is not transported by TAP into the ER.

In summary, there is no question that TAP preselects peptides based upon peptide length. Peptides that are the optimal size for binding to class I molecules, i.e., 8–12 aa, are transported with the greatest efficiency, while longer peptides are transported at a reduced efficiency. It is also clear that mouse and rat TAP preselect peptides based upon the C-terminal residue; however, it is not clear how selective the human transporter is. While a peptide-binding motif for human TAP has been described, its true extent and significance needs to be explored in more detail. In humans, TAP plays the least important role in determining which peptides are presented to T cells. Human TAP provides an abundant supply of peptides that are the optimal size for binding to class I molecules, and it is the class I molecule that provides the ultimate specificity. Mouse and rat TAP play a more important role in peptide selection due to their preference for peptides that contain hydrophobic

C termini. This is why mouse class I peptide-binding motifs contain hydrophobic anchor residues at the C terminus.

New data with regard to substrate specificity and peptide binding to TAP have recently emerged. In one study, four polymorphic amino acid residues that contribute to the specificity of the rat cim^a and cim^b transporters were identified (Momburg et al., 1996). Chimeric rat TAP2 molecules were made between segments of the TAP2^a and TAP2^u genes that encoded groups of the polymorphic residues. These chimeric molecules were then expressed along with rat TAP1 and assayed for peptide transport using iodinated peptides in which the C-terminal amino acid was varied. This allowed for the identification of the particular polymorphic residues that contribute to the C-terminal amino acid selectivity of rat TAP. Four polymorphic residues were identified in two separate regions of rat TAP2 (residues 217/218 and 374/380), which map to the two putative cytoplasmic loops closest to the hydrophilic C-terminal ATP-binding domain, and which appear to be located very close to the ER membrane. It is thought that the residues identified, though spatially separate, come together to form part of the peptide-binding pocket.

In a second study (Nijenhuis et al., 1996), photoreactive peptide analogs were used to identify a segment of human TAP1 that appears to be involved in peptide binding. The photoreactive peptides contained a photoreactive analog of phenylalanine. The photopeptides were transported normally by TAP, and therefore should interact with the same residues on TAP as wild-type peptide. The results of the photolabeling experiments revealed that both TAP1 and TAP2 were labeled by the photoreactive peptides (Nijenhuis et al., 1996), suggesting that the peptide-binding site of TAP is made up of elements of both TAP1 and TAP2 (Androlewicz et al., 1994). Individual chains expressed separately were not photolabeled, indicating that both TAP1 and TAP2 are required to form a functional peptide-binding site. In addition, it was shown that both TAP1 and TAP2 chains were labeled regardless of the position of the photoreactive group within the peptide. However, the ratio of TAP1 to TAP2 labeling varied between the peptides. It was suggested that the orientation of the photopeptide within the binding site most likely determined which chain was predominantly labeled. The photolabeling approach was taken one step further by identifying the region of TAP1 that is involved in peptide binding (Nijenhuis et al., 1996). This was made possible by digesting the photolabeled TAP samples with trypsin, and identifying the tryptic fragments with antisera raised against specific TAP1 peptides. Thus, it was possible to map the site of peptide binding to a segment of TAP1 that comprises the two transmembrane domains closest to the cytosolic ATP-binding domain. This site corresponds to a similar region involved with substrate binding on P-glycoprotein, a homologous ABC transporter involved in multidrug resistance (Bruggemann et al., 1992).

An exciting new area of research in the class I processing pathway is the identification of molecules that associate with TAP and possibly modulate its function. Heterodimers of class I heavy chain and β_2-microglobulin were shown to associate physically with TAP in coimmunoprecipitation experiments (Ortmann et al., 1994; Suh et al., 1994). In addition, recent evidence suggests that class I interaction with TAP may be necessary to load class I molecules with peptide (Grandea et al., 1995; Peace-Brewer et al., 1996). It is thought that the physical interaction with TAP facilitates peptide loading by localizing the class I molecules to the site of peptide translocation. Another possibility is that association with class I enhances the rate of peptide transport by TAP. Whatever the effect TAP/class I interaction has on peptide loading, it does not appear to be critical because class I molecules can be loaded effectively in TAP-negative cells when peptides are introduced into the ER through a leader sequence (Anderson et al., 1991). Also, it appears that TAP efficiently transports peptides in the absence of class I molecules (Grandea et al., 1995; Meyer et al., 1994), suggesting that interaction with class I molecules is not required for TAP to function. There is evidence that another protein, of molecular mass 48,000, associates with TAP (Ortmann et al., 1994; Peace-Brewer et al., 1996). The physiological role, if any, of the 48 kDa protein remains to be determined, but the evidence indicates that it may be involved in mediating class I association with TAP (Grandea et al., 1995). Thus, the number of players involved in the assembly and loading of MHC class I molecules is increasing, and the true significance of their roles needs further study.

These recent studies have provided information on the molecular mechanism of peptide transport by TAP. By identifying sites that are involved with peptide binding, and identifying molecules that associate with TAP, we are closer to an understanding of how TAP works. However, unanswered questions remain with regard to the exact nature of the TAP–peptide interaction, the role of associated proteins, and the mechanism of peptide translocation across the membrane. We await with interest future information that will provide insight into the mechanism of action of this important transporter.

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